RM#9176 CD# 1998/081

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/55, 31/41, 31/195, 31/40, 31/485 — A1

(11) International Publication Number: WO 98/30220

(43) International Publication Date: 16 July 1998 (16.07.98)

(21) International Application Number: PCT/US98/00006

(22) International Filing Date: 6 January 1998 (06.01.98)

(30) Priority Data:
08/781,814 9 January 1997 (09.01.97) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 08/781,814 (CON)
Filed on 9 January 1997 (09.01.97)

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: ATTENUATION OF OPIOID TOLERANCE BY INHIBITING INDUCIBLE NITRIC OXIDE SYNTHASE PATHWAYS IN THE TREATMENT OF PAIN

(57) Abstract

Inducible nitric oxide (iNOS) inhibitors are used to prevent and/or reverse tolerance that is seen upon prolonged usage of opioids in the clinical management of moderate to severe pain.

RTS-0066

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ATTENUATION OF OPIOID TOLERANCE BY INHIBITING INDUCIBLE NITRIC OXIDE SYNTHASE PATHWAYS IN THE TREATMENT OF PAIN

Field of the Invention

The present invention is directed to the use of inducible nitric oxide (iNOS) inhibitors in preventing and/or reversing tolerance that is seen upon prolonged usage of opioids in the clinical management of moderate to severe pain.

Background of the Invention

The major opiate drugs, such as morphine, are widely used as analgesics in the clinical management of severe chronic pain. These drugs act on pain perception within the central nervous system in the clinical management of pain originating in the viscera or arising from severe injuries, burns, and neoplasms. Major opiate drugs that are used therapeutically to relieve severe pain seem to produce most of their analgesic actions through interactions with the μ type receptor.

Tolerance to the analgesic effects of opioids occurs upon prolonged usage. Well documented clinical example of this is seen in cancer patients. The net result of tolerance is decreased pain relief, increased side effects, and decreased clinical efficacy. Narcotic-induced hyperalgesia (NIH) may also develop in association with the tolerance of the opioid. This limits the clinical usefulness of these opiates in patients who require long term use of this opiate to relieve pain.

For instance, morphine is administered to a patient to relieve severe cancer pain. As the disease progresses, the intensity of the pain increases, and the morphine dosage increases. Tolerance develops and there is a decrease in analgesic effects. Thus the morphine dosage increases. Side effects develop including respiratory depression, vomiting, constipation, confusion, and immune system depression. There is an overall decrease in clinical efficacy

There has been no adequate strategy to overcome clinical development of tolerance and hence to provide an appropriate approach for the treatment of severe and chronic pain. One strategy used in the clinical management of opioid analgesic tolerance involves switching the type of opioid used on the assumption that receptor

profiles of the commonly used opioid are dissimilar enough to reduce cross tolerance. Unfortunately the outcome of this action is not very satisfactory.

The mechanisms underlying opiate tolerance is poorly understood but it is known to involve the NMDA receptor since the NMDA receptor antagonist MK-801 has been shown in rats to prevent morphine tolerance. (Trujillo 1991). NMDA stimulates nitric oxide synthase (NOS) the enzyme responsible for the biosynthesis of nitric oxide (NO). Three isoforms of NOS have been identified to date. Two are constitutively expressed and are in general responsible for the beneficial effects described so far to NO. These are the endothelial and neuronal isoforms of NOS (eNOS and nNOS). The third isoform is inducible (iNOS) and accounts for the detrimental effects that have been observed for NO in several pathological conditions. Induction of iNOS is blocked by steroids such as dexamethasone. NOS is observed immunohistochemically in amygdala, grey matter, substantia gelatinosa of the spinal cord. These regions contain opioids receptors and are areas important in the control of the pain response. There is evidence from the literature that non-selective NOS inhibitors such as N^G-nitro arginine (NO_2Arg) prevents and reverses morphine-induced tolerance.

Taken together, these findings highlight the possible role of NO in the development of morphine-induced tolerance. Inhibition of NOS by non-selective NOS inhibitors such as NO_2Arg is associated with a vast array of detrimental side effects. To name but a few, these include: increased blood pressure and hypertension, increased platelet and white blood cell reactivity, decreased cerebral blood flow, gastrointestinal and renal toxicity.

Another problem with the use of opioids is severe physical dependence on the opioid which makes withdrawal very unpleasant. This makes it difficult for the clinician to take patients off the opioid when pain relief occurs or when an alternative route of pain relief is necessary because of the fear of eliciting unpleasant side effects originating from the abrupt removal of the opioid. Clonidine and buprenorphine are used in the clinic to attenuate the symptoms of withdrawal. However, these have their own side effects. For instance, clonidine causes hypotension and its effectiveness is limited by the susceptibility of the patient to this effect and buprenorphine has opioid

agonist property which provides for potential abuse of the drug. It is known that non-selective NOS inhibitors such as NO_2Arg will prevent or relieve withdrawal symptoms associated with morphine withdrawal. (Bhargana, H.N., "Attenuation of Tolerance to and Physical Dependence on Morphine in the Rat by Inhibition of Nitric Oxide Synthase," *Gen. Pharmac.*, 26, 1049-1053, 1995) Nevertheless, as stated above, these drugs have a number of serious side effects limiting their therapeutic uses.

It is desired to find an effective means to prevent tolerance to opioids in the treatment of pain and to relieve side effects of withdrawal without the adverse effects of known treatments.

Summary of the Invention

The present invention is directed to a method for preventing opioid tolerance in a human or animal subject. In accordance with this method, an effective amount of a suitable opioid and an effective amount of at least one iNOS inhibitor is administered to the subject to prevent tolerance to the opioid.

The present invention is also directed to a method for attenuating the symptoms of opioid withdrawal in a human or animal subject. In accordance with this method, an effective amount of at least one iNOS inhibitor is administered to the subject for a period of time effective to attenuate the symptoms upon opioid withdrawal.

The present invention is further directed to a method of preventing or eliminating hyperalgesia induced by opioid. In accordance with this method, an effective amount of at least one iNOS inhibitor is administered to the subject to eliminate or prevent hyperalgesia.

In addition, the present invention is directed to a pharmaceutical composition containing an opioid; an iNOS inhibitor; and a pharmaceutically acceptable carrier.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the present invention as claimed.

Brief Description of the Drawings

Fig. 1 depicts the inhibition of morphine-induced tolerance in mice by NO_2Arg.

Fig. 2 depicts the inhibition of morphine-induced tolerance in mice by iNOS inhibitors.

Fig. 3 depicts the inhibition of reduced analgesic effects of morphine in morphine-induced tolerant mice by an iNOS inhibitor.

Fig. 4 depicts the prevention of loss of analgesic effects of morphine in morphine-tolerant mice by iNOS inhibitors.

Fig. 5 depicts the effects of an iNOS inhibitor on morphine-induced tolerance in mice.

Fig. 6 depicts the reversal of morphine-induced tolerance by NO_2Arg.

Fig. 7 depicts the reversal of morphine-induced tolerance by iNOS inhibition.

Fig. 8 depicts the acute effect of iNOS inhibition to reverse morphine tolerance (higher doses).

Fig. 9 depicts the acute effect of iNOS inhibition to reverse morphine tolerance (lower doses).

Detailed Description of the Invention

The present invention relates to the use of iNOS inhibitors for the treatment and prevention of opioid tolerance. For instance, the dual administration of an iNOS inhibitor together with morphine in cancer patients allows lower doses of the morphine to elicit its analgesic effects while limiting its side effects. Moreover, an iNOS inhibitor can reverse opioid tolerance in patients that have already developed such tolerance. Thus the selective iNOS inhibitors restore the loss of analgesic effects observed upon prolonged treatment with an opioid. Furthermore, the selective inhibition of iNOS inhibitors do not have the side effects associated with non-selective NOS inhibitors.

The iNOS inhibitors themselves are not analgesic, nor do they potentiate the acute analgesic effects of morphine in non-tolerant animals. Acute administration of an iNOS inhibitor, once tolerance has developed, will restore the loss of analgesic effect of this opiate. The iNOS inhibitors at low doses attenuate opioid tolerance without affecting the antinocicoptive effects of morphine. This indicates selective action upon mechanisms involved with tolerance rather than simple potentiation of morphine. Thus in the cancer patient, if the iNOS inhibitor is administered with the

morphine, there is no tolerance and analgesic effect is maintained. Consequently there is no need for an increase in morphine dose, there is reduced side effects, and there is an increase in clinical efficacy. In addition, iNOS inhibitors have no hemodynamic effect

Further, iNOS inhibitors will eliminate or prevent hyperalgesia associated with morphine use. Hyperalgesia is the enhanced nociceptive response to an otherwise non-noxious response and develops in association with the development of morphine tolerance. Both tolerance and hyperalgesia share an active, crucial role of activation of glutamatergic neurotransmission. Therefore, a subject treated with a narcotic may perceive more pain, in part due to the development of narcotic-induced hyperalgesia (NIH), in response to a preexisting painful condition even though there is no substantial increase in the intensity of peripheral factors causing pain. Thus progressively higher narcotic doses are needed to overcome the loss of analgesic effects of narcotics due to the development of both tolerance and NIH. In turn a vicious circle is initiated involving higher narcotic doses, more tolerance, greater NIH.

The use of an iNOS inhibitor will break the vicious circle and furthermore, will prevent hyperalgesia from occurring. Thus the present invention further relates to the use of iNOS inhibitors for the treatment and prevention of hyperalgesia.

The iNOS inhibitor of the present invention can be used with opioids in the treatment of many types of pain. Pain states include pain associated with tumor infiltration, e.g. bone and nerve, pain associated with cancer therapy, e.g. postsurgical syndromes, postchemotherapy syndromes, postradiation syndromes, neurophatic pain of diverse nature, acute postoperative pain, and acute pain associated with inflammatory response.

Based on data obtained, it is reasonable to expect that any iNOS inhibitor would work in the present invention. All iNOS inhibitors are envisioned to reduce or eliminate opioid tolerance and associated hyperalgesia, and to eliminate withdrawal symptoms. These iNOS inhibitors include those recited in related applications U.S. Serial Nos. 08/139,970, 08/141,168, 08/448,473, 08/635,188, 08/209,094, 08/218,160, 08/336,956, 08/425,831, and 08/438,321 and PCT applications 94/11724, 94/11832, 95/02669, 94/03589, 95/14001, 96/05315, and 96/06836 which are hereby

incorporated by reference in their entirety. Also included are iNOS inhibitors disclosed in other works such as Merck & Co., Inc. including WO 96/18616, WO 96/14842, and WO 96/14842 and Abbott Laboratories including WO 94/12163 which are all incorporated by reference in their entirety.

Pharmaceutically acceptable salts, in particular acid addition salts, of the iNOS compounds are also contemplated for use in the present invention. Suitable salts include those formed with both organic and inorganic acids. Suitable salts are recognized in the Searle, Merck and Abbot applications and are hereby incorporated by reference in their entirety.

The iNOS inhibitors useful in the present invention may be used alone, in combination with one another, or in combination with other classes of drugs useful in preventing tolerance to opioids or useful in attenuating the opioid withdrawal syndrome.

Preferably the iNOS inhibitor is administered as a pharmaceutical formulation containing one or more pharmaceutically acceptable carriers. The carriers should be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof. Suitable carriers include, but are not limited to, distilled water, saline and acidified saline.

The formulations include those suitable for oral, parenteral (including subcutaneous, intradermal, intramuscular, intravenous, and intra articular), rectal and topical (including dermal, buccal, sublingual, and intraocular) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of an active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary, or paste.

Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain antioxidants, buffers, bacteriostats and

solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous sterile suspensions which may include suspending agents and thickening agents.

Formulations for rectal administration may be presented as a suppository with the usual carriers such as cocoa butter or polyethylene glycol. Formulations for topical administration in the mouth, for example buccally or sublingually, include lozenges and pastilles.

In addition to the ingredients particularly mentioned above, the formulations may include other agents conventional in the art.

Preferred unit dose formulations are those containing an effective dose, as hereinbelow recited, or an appropriate fraction thereof, of the active ingredient.

The iNOS inhibitors may be administered orally or via injection at a dose of from 0.1 to 300 mg/kg per day. The dose range for adult humans is generally from 5 mg to 500 mg/day. Tablets or other forms of presentation provided in discrete units may conveniently contain an amount of compound of the invention which is effective at such dosage or as a multiple of the same, for instance, units containing 5 mg to 500 mg, usually around 10 mg to 200 mg.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

It will be understood that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diets, time of administration, rate of excretion, drug combination, and the severity of the particular disease undergoing therapy.

The iNOS inhibitors are preferably administered orally or by injection (intravenous or subcutaneous). The precise amount of the iNOS inhibitor administered to a patient will be the responsibility of the attending physician. However, the dose employed will depend on a number of factors, including the age and sex of the patient, and the particular opioid being used. The route of administration may also vary.

The iNOS compound can be administered with the opioid as a single bolus or in several doses. The dosage plan will depend on the factors discussed above, such as intensity of pain, age and sex of patient. A typical dosage may be twice a day.

The following examples are for purposes of illustration and are not intended to limit the scope of the claimed invention.

Examples

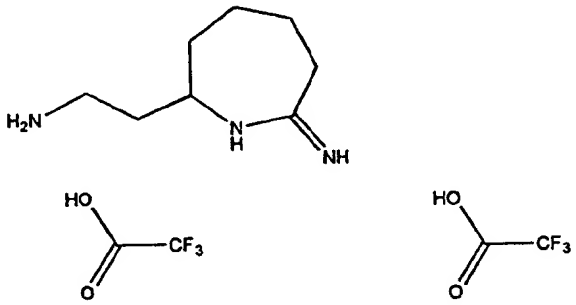
In the following examples, the following compounds were used as indicated.

Non-selective NOS inhibitor

NO_2Arg N^G-monomethyl-L-arginine

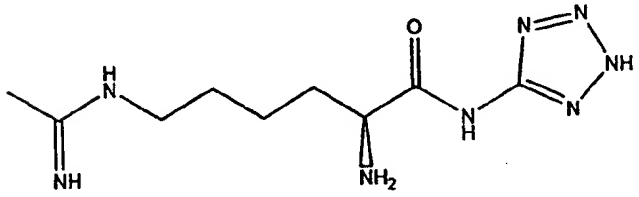
Inducible NOS (iNOS) inhibitor

Compound A



Hexahydro-7-imino-1H-azepine-2-ethanamine, trifluoro acetate salt

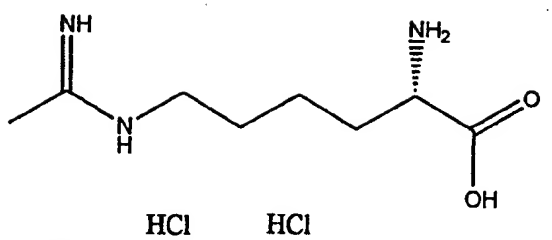
Compound B



HCl HCl

2S-amino-6-[(1-iminoethyl)amino]-N-(1H-tetrazole-5-yl) hexanamide, hydrate, dihydrochloride

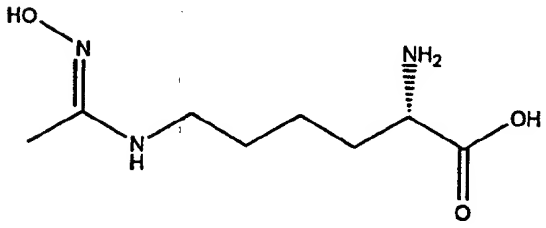
Compound C



HCl HCl

N^6-iminoethyl-L-lysine, dihydrochloride

Compound D

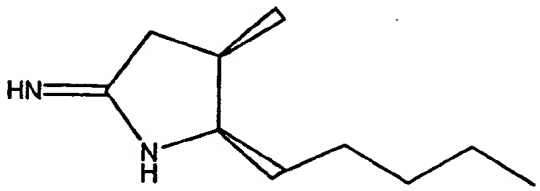


HCl

HCl

N^6-[1-(hydroxyimino)ethyl-L-lysine, dihydrochloride

Compound E



HCl

4-methyl-5R-pentylpyrrolidin-2-imine, monohydrochloride

Example 1

Mice were treated twice a day with either saline (naive) or morphine (s.c, 10 mg/kg) for a period of 4 days in order to induce tolerance (Tol). On day 5, all mice received a s.c. challenge dose of morphine (3 mg/kg) and analgesia measured 30 min. later. Dose response to morphine had indicated that a challenge dose of 3 mg/kg normally elicits a 90% analgesia in naive non-tolerant mice when assessed by standard hot plate test. As can be seen from this example, mice that were treated with morphine for the 4 days period responded with a lessened analgesic response to morphine when compared to the naive mice (this loss of analgesia is referred to as tolerance). Tolerance to morphine was restored in mice that were treated with the non-selective NOS inhibitor NO_2Arg. NO_2Arg was given intraperitoneally (i.p) twice a day at 4 mg/kg before each morphine injection. The total number of mice in each group was 20.

Example 2

Mice were made tolerant to morphine as described in Example 1. One set of mice was treated with NO_2Arg (4 mg/kg i.p twice a day for 4 days before each sc morphine injection), the other two groups with the selective iNOS inhibitors Compounds A and B, (3 mg/kg i.p twice a day before each sc morphine injection). The iNOS inhibitors prevented morphine-induced tolerance. The total number of mice in each group was 20.

Example 3

Mice were made tolerant to morphine as described in Example 1. Mice were treated with different doses of the selective iNOS inhibitor Compound A (1 to 8 mg/kg twice a day for 4 days with each dose given before each sc morphine injection). Compound A prevented in a dose-dependent manner the development of tolerance. The total number of mice in each group was 20.

Example 4

Mice were made tolerant to morphine as described in Example 1. Shows inhibition of morphine induced tolerance to the analgesic effects of morphine with the iNOS inhibitors Compounds A, B, C, and D. The iNOS inhibitors were given i.p at 4 mg/kg twice a day (each dose before each morphine injection) for 4 days. Example also shows that much higher doses of the less selective iNOS inhibitors aminoguanidine (AG) was required to inhibit morphine induced tolerance (10 or 20 mg/kg a day twice a day for 4 days with each dose given before each morphine injection). The total number of mice in each group was 20.

Example 5

Mice were made tolerant to morphine as described in Example 1. Shows inhibition of the reduced analgesic effects of morphine in morphine induced tolerant mice by an iNOS inhibitor Compound E. the iNOS inhibitor was given i.p at 4 mg/kg twice a day (each dose given before each morphine injection) for 4 days. The total number of mice in each group was 20.

Example 6

Shows reversal by NO_2Arg of established loss of analgesia to morphine in mice made tolerant to morphine. Two groups of mice were made tolerant to morphine by s.c dosing of morphine at 10 mg/kg twice a day for a period of 13 days. Control mice received (instead of morphine) a twice daily injection of saline (referred to as naive). Analgesic response to the usual challenge dose of morphine (3 mg/kg) was measured every other day for the total period of study (13 days) in every mouse. At day 5, it is clear that all mice (except naive) were tolerant to the analgesic effects of morphine. At this time point, one of these groups received i.p injections of NO_2Arg (4 mg/kg twice a day with each dose given before each morphine injection) for the next 4 days. Analgesia was measured by hot plate every 2 days. It is clear that NO_2Arg reverses established tolerance to the analgesic effects of morphine. On day 9, the NO_2Arg was stopped and analgesia measured every 2 days until day 13. Reversal of tolerance by

NO_2Arg is lost when the treatment was stopped. The total number of mice in each group was 20.

Example 7

The iNOS inhibitor Compound A reversed the established loss of analgesia to morphine in mice made tolerant to morphine. The protocol and drug dosing regimen used in this example was identical to that described in Example 6. Compound A reverses established tolerance to the analgesic effects of morphine. Reversal of tolerance is lost upon removal of this selective iNOS inhibitor. The total number of mice in each group was 20.

Example 8

This example shows the acute effect of iNOS inhibition to reverse morphine tolerance.

Part 1. Mice were treated twice a day with either saline (naive) or morphine (s.c., 10 mg/kg) for 12 days to induce and maintain tolerance. On days 5, 8, and 12, several doses (ip, mg/kg) of Compound E (A 0.4, B 1.0, C 4.0), Compound A (D 1.0, E 4.0) and NO_2Arg (F 4.0) were injected 40 min. before a s.c. challenge dose of 3.0 mg/kg morphine. % Analgesia was measured 50 min. later. The total number of mice in each group was 10-20. The results are shown Figure 8.

Part 2. Mice were treated in the same way as part 1 except on days 5, 8, and 12, lower acute doses (ip, mg/kg) of Compound E (G 0.04, H 0.1) and Compound A (I 0.04, J 0.1) were injected. The effect of Compounds E and A was dose dependent. The results are shown in Figure 9.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

What is Claimed:

1. A method for preventing opioid tolerance in a mammal, comprising administering to said mammal, an effective amount of a suitable opioid and an effective amount of at least one iNOS inhibitor to prevent tolerance to the opioid.

2. The method of claim 1 wherein the opioid is morphine.

3. The method of claim 1 wherein the iNOS inhibitor is selected from the group consisting of hexahydro-7-imino-1H-azepine-2-ethanamine, 2S-amino-6-[(1-iminoethyl)amino]-N-(1H-tetrazole-5-yl) hexanamide, N^6-iminoethyl-L-lysine, N^6-[1-(hydroxyimino)ethyl-L-lysine, dihydrochloride, 4-methyl-5R-pentylpyrrolidin-2-imine, and pharmaceutically acceptable salts thereof.

4. The method of claim 1 in which said administering is conducted orally, intramuscularly, subcutaneously, transdermally, intravenously, or intra peritoneally.

5. The method of claim 1 wherein the iNOS inhibitor is administered in a pharmaceutically acceptable carrier.

6. The method of claim 5 wherein the pharmaceutically acceptable carrier comprises distilled water, saline or acidified saline.

7. The method of claim 5 wherein the pharmaceutically acceptable carrier contains at least one selected from the group consisting of antioxidants, stabilizers, buffers, bacteriostats, suspending agents, and thickening agents.

8. The method of claim 1 wherein the effective amount of the iNOS inhibitor is about 0.1 to 300 mg/kg per day.

9. A method for attenuating the symptoms of opioid withdrawal in a mammal, comprising administering to said mammal, an opioid withdrawal symptom attenuating amount of iNOS inhibitor for a period of time effective to attenuate said symptoms upon opioid withdrawal.

10. The method of claim 9 wherein the opioid is morphine.

11. The method of claim 9 wherein the iNOS inhibitor is selected from the group consisting of hexahydro-7-imino-1H-azepine-2-ethanamine, 2S-amino-6-[(1-iminoethyl)amino]-N-(1H-tetrazole-5-yl) hexanamide, N^6-iminoethyl-L-lysine, N^6-[1-

(hydroxyimino)ethyl-L-lysine, dihydrochloride, 4-methyl-5R-pentlypyrrolidin-2-imine, and pharmaceutically acceptable salts thereof.

12. The method of claim 9 in which said administering is conducted orally, intramuscularly, subcutaneously, transdermally, intravenously, or intra peritoneally.

13. The method of claim 9 wherein the iNOS inhibitor is administered in a pharmaceutically acceptable carrier.

14. The method of claim 13 wherein the pharmaceutically acceptable carrier comprises distilled water, saline or acidified saline.

15. The method of claim 13 wherein the pharmaceutically acceptable carrier contains at least one selected from the group consisting of antioxidants, stabilizers, buffers, bacteriostats, suspending agents, and thickening agents.

16. The method of claim 9 wherein the effective amount of the iNOS inhibitor is about 0.1 to 300 mg/kg per day.

17. A method of preventing or eliminating hyperalgesia induced by opioid use by administering to a human or animal subject an effective amount of at least one iNOS inhibitor to eliminate or prevent hyperalgesia.

18 a method for reversing opioid tolerance in a mammal, comprising administering to said mammal, an effective amount of at least one iNOS inhibitor to reverse tolerance to the opioid.

19. The method of claim 18 wherein the opioid is morphine.

20. The method of claim 18 wherein the iNOS inhibitor is selected from the group consisting of hexahydro-7-imino-1H-azepine-2-ethanamine, 2S-amino-6-[(1-iminoethyl)amino]-N-(1H-tetrazole-5-yl) hexanamide, N^6-iminoethyl-L-lysine, N^6-[1-(hydroxyimino)ethyl-L-lysine, dihydrochloride, 4-methyl-5R-pentlypyrrolidin-2-imine, and pharmaceutically acceptable salts thereof.

21. The method of claim 18 in which said administering is conducted orally, intramuscularly, subcutaneously, transdermally, intravenously, or intra peritoneally.

22. The method of claim 18 wherein the iNOS inhibitor is administered in a pharmaceutically acceptable carrier.

23. The method of claim 22 wherein the pharmaceutically acceptable carrier comprises distilled water, saline or acidified saline.

24. The method of claim 22 wherein the pharmaceutically acceptable carrier contains at least one selected from the group consisting of antioxidants, stabilizers, buffers, bacteriostats, suspending agents, and thickening agents.

25. The method of claim 18 wherein the effective amount of the iNOS inhibitor is about 0.1 to 300 mg/kg per day.

26. a pharmaceutical composition comprising:

a) an opioid;

b) an iNOS inhibitor; and

c) a pharmaceutically acceptable carrier.

27. The composition of claim 26 wherein the opioid is morphine.

28. The composition of claim 26 wherein the iNOS inhibitor is selected from the group consisting of hexahydro-7-imino-1H-azepine-2-ethanamine, 2S-amino-6-[(1-iminoethyl)amino]-N-(1H-tetrazole-5-yl) hexanamide, N^6-iminoethyl-L-lysine, N^6-[1-(hydroxyimino)ethyl-L-lysine, dihydrochloride, 4-methyl-5R-pentlypyrrolidin-2-imine, and pharmaceutically acceptable salts thereof.

29. The composition of claim 26 wherein the pharmaceutically acceptable carrier comprises distilled water, saline or acidified saline.

30. The composition of claim 26 wherein the pharmaceutically acceptable carrier contains at least one selected from the group consisting of antioxidants, stabilizers, buffers, bacteriostats, suspending agents, and thickening agents.

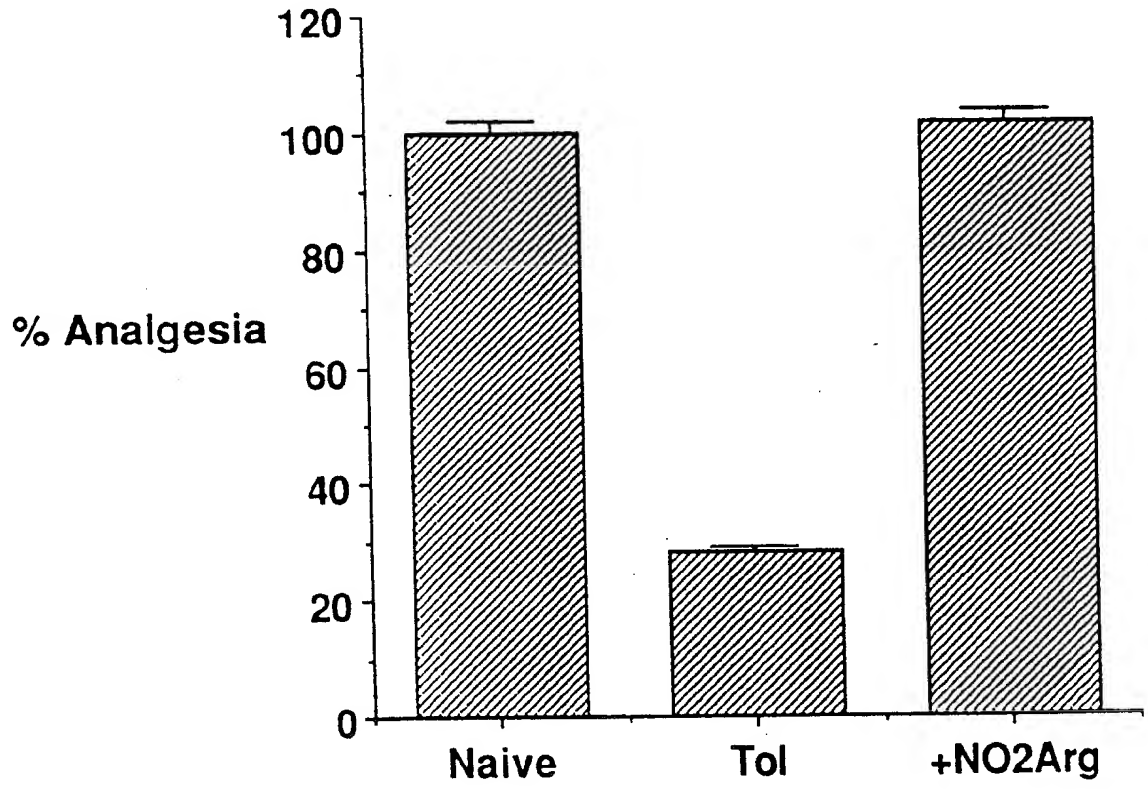


Fig. 1

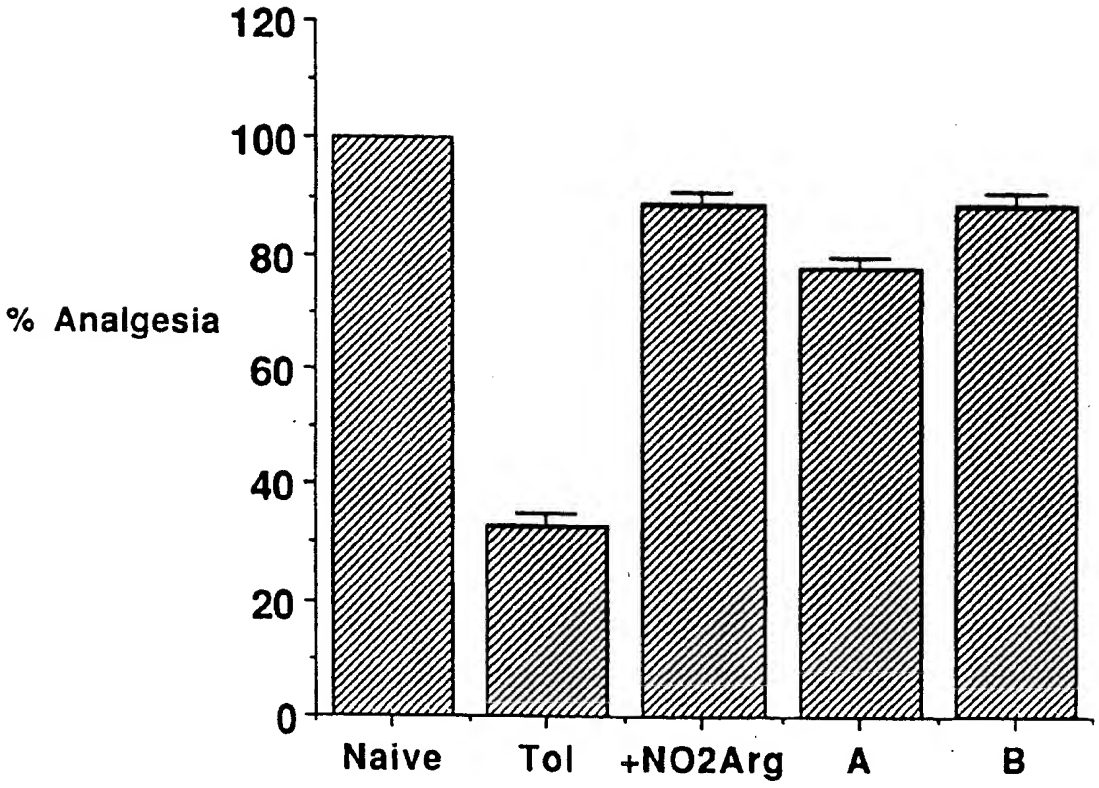


Fig. 2

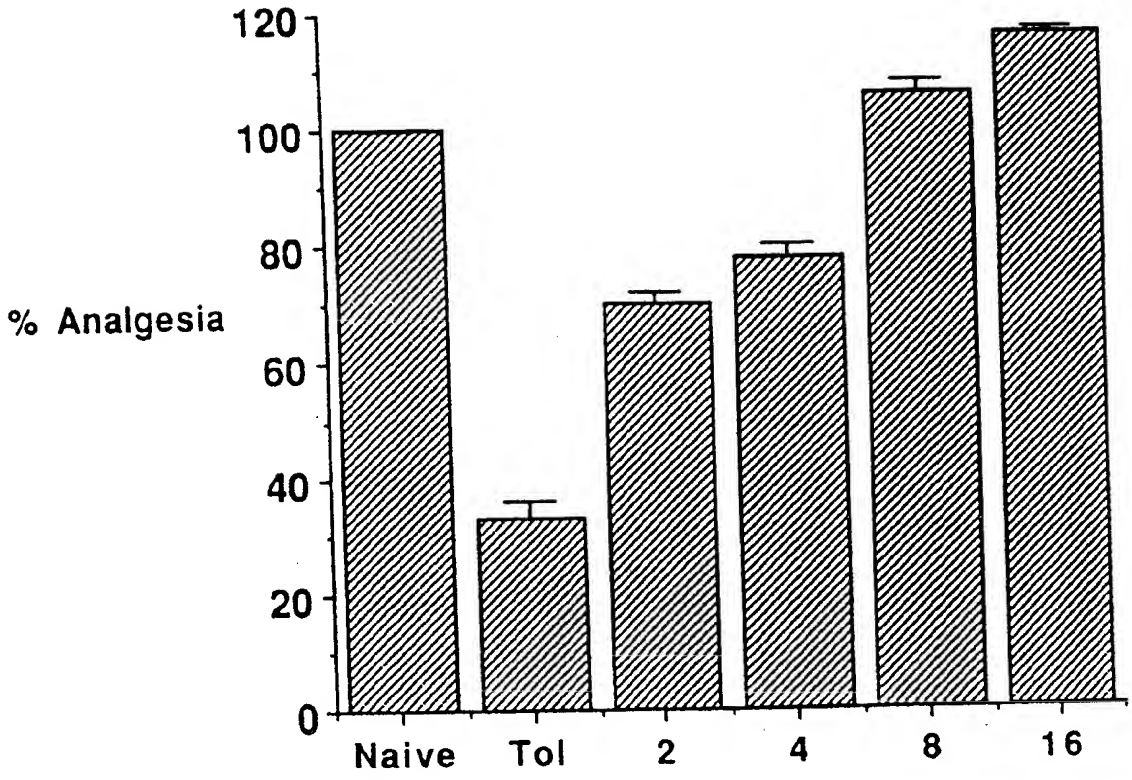


Fig. 3

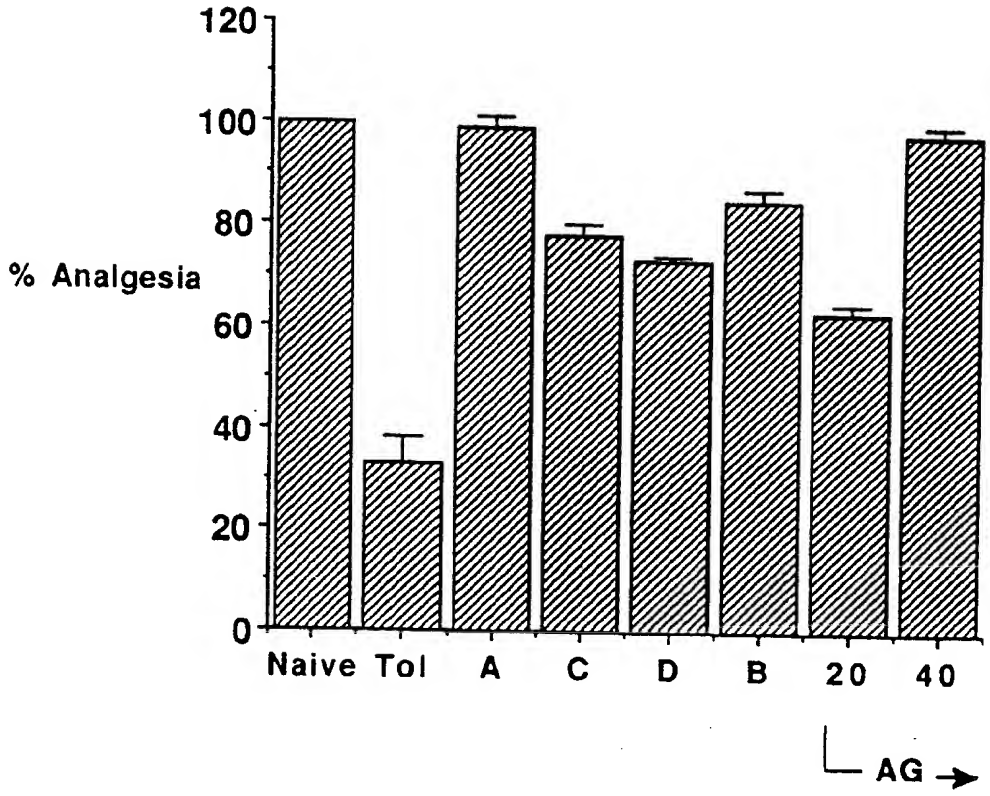


Fig. 4

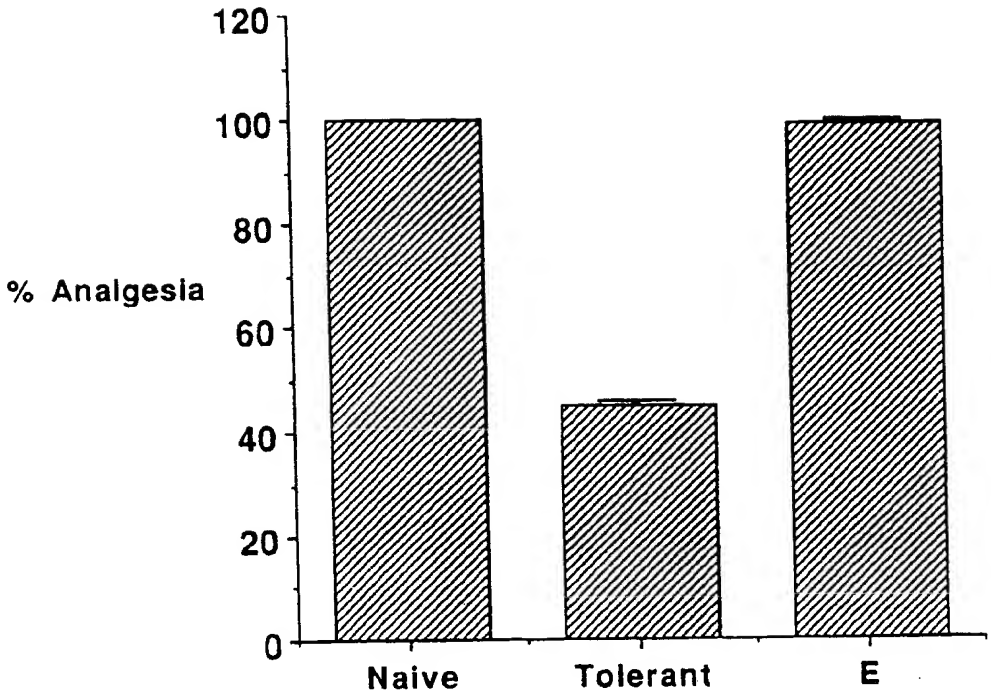


Fig. 5

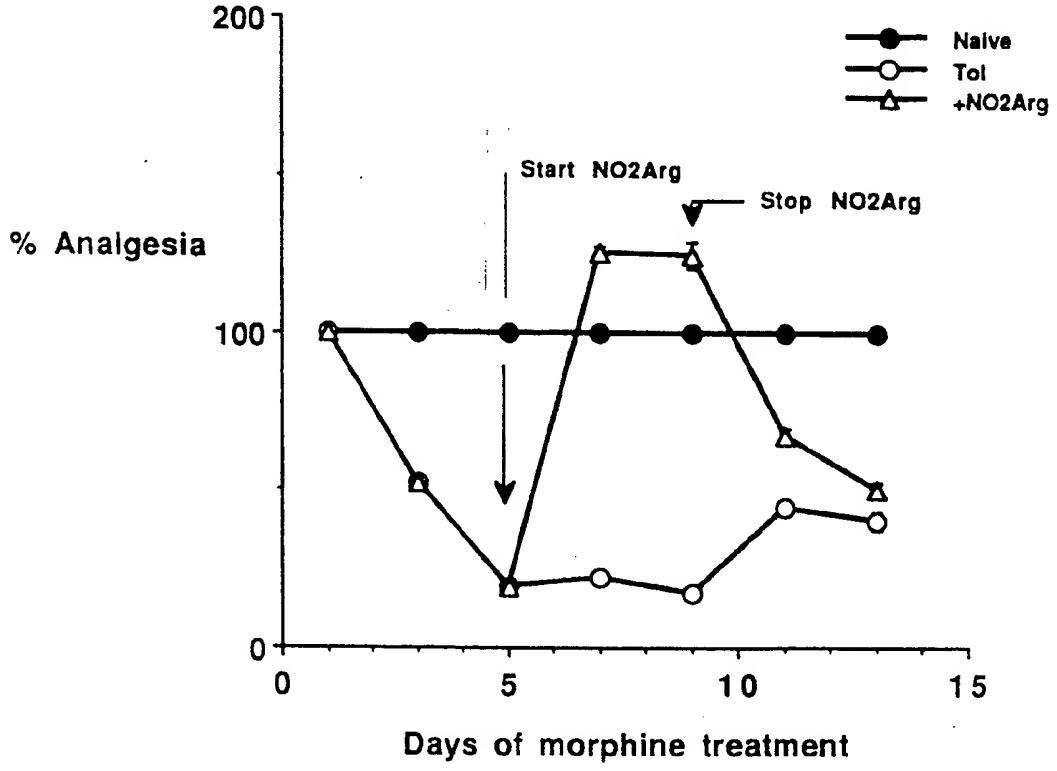

200
100
0
% Analgesia
Naive
Tol
+NO2Arg
Start NO2Arg
Stop NO2Arg
0
5
10
15
Days of morphine treatment

Fig. 6

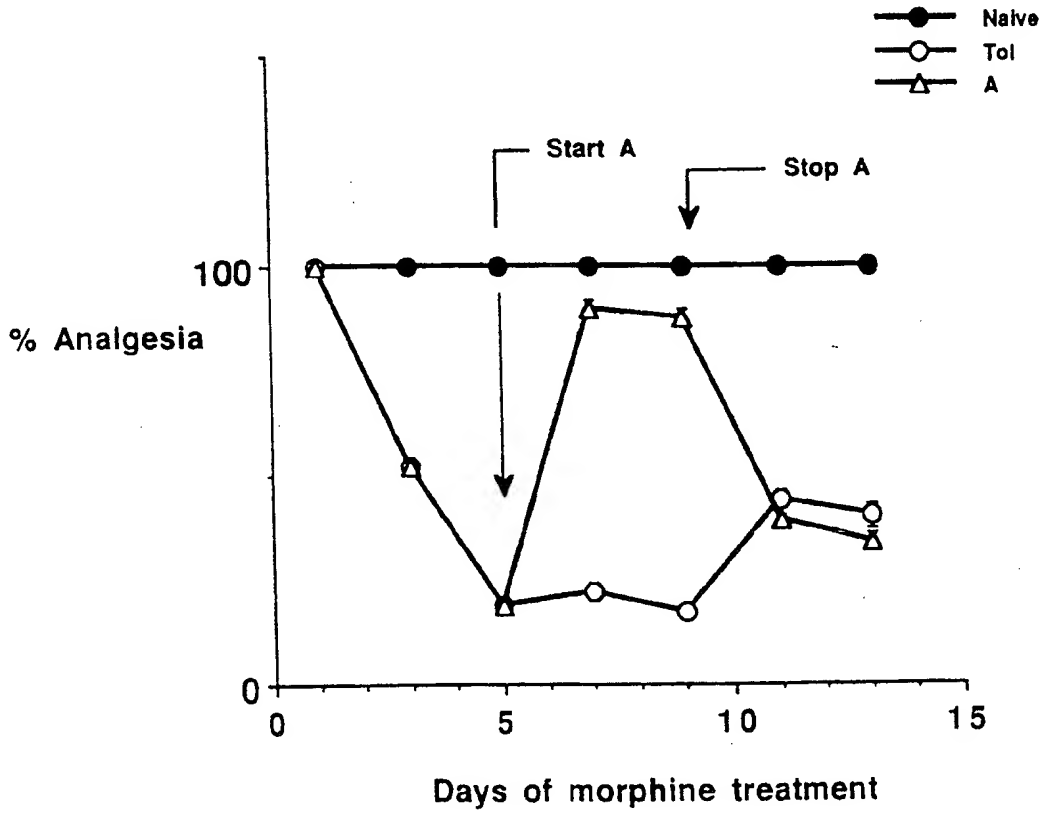

Naive
Tol
A
Start A
Stop A
100
% Analgesia
0
0
5
10
15
Days of morphine treatment

Fig. 7

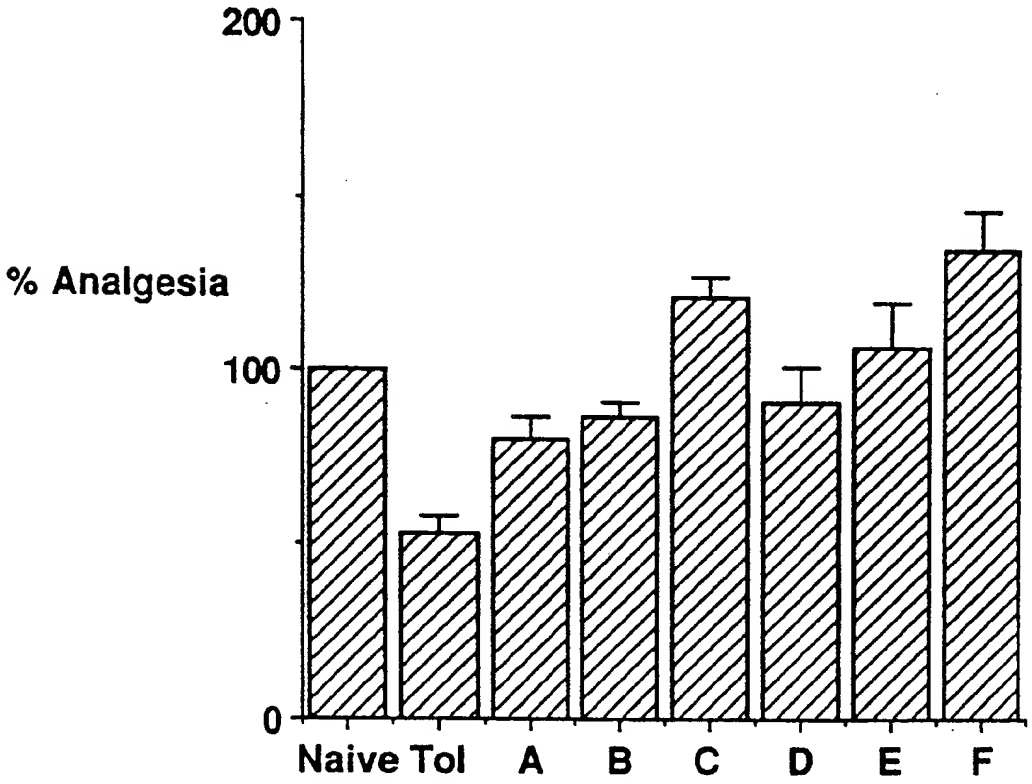


Fig. 8

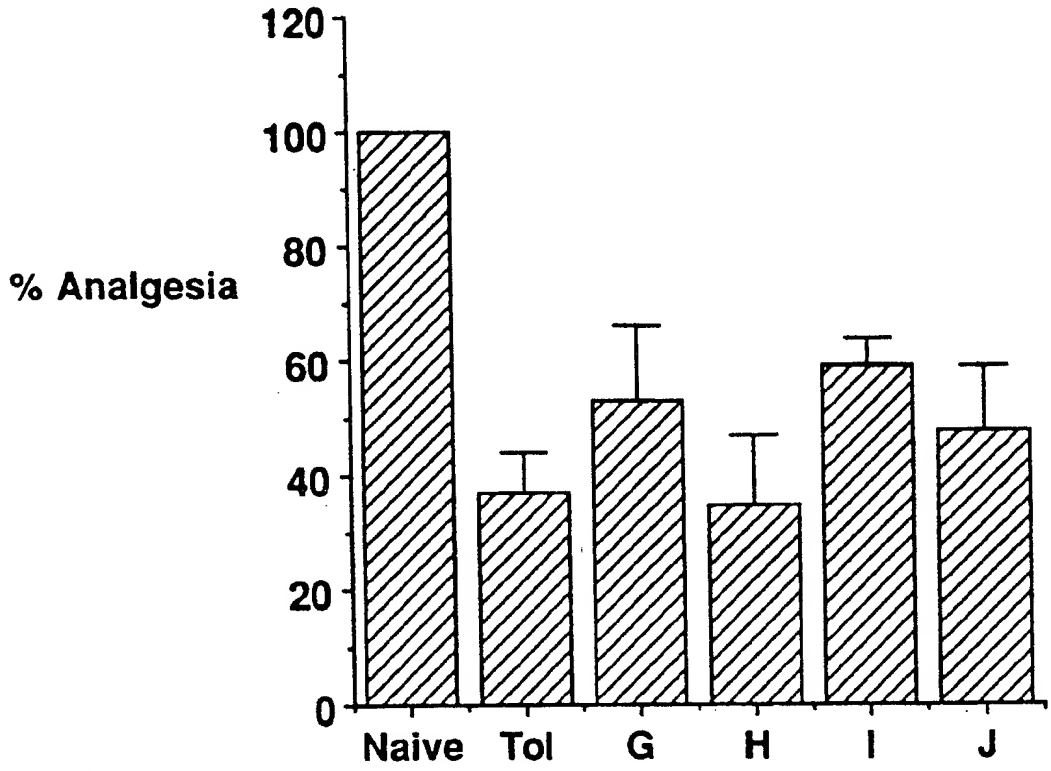


Fig. 9

INTERNATIONAL SEARCH REPORT

	national Application No PCT/US 98/00006

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/55 A61K31/41 A61K31/195 A61K31/40 A61K31/485

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 35677 A (SEARLE & CO) 14 November 1996 see page 8, line 15-30; claims 1,8,11 see page 8, line 23-27 ---	1,3,5,8, 9,11,13, 16-18, 20,22, 25,26,28
X	WO 96 18616 A (MERCK & CO) 20 June 1996 cited in the application see page 6, line 5-9 see page 20, line 2-5; claims 14,15 see page 24, line 10-19; table 2 --- -/--	1,4-9, 12-18, 21-26, 29,30

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 May 1998	11. 06. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kanbier, D

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/00006

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 321 012 A (VIRGINIA COMMONWEALTH UNIVERSITY MEDICAL COLLEGE) 14 June 1994	1,2,4-8, 26,27, 29,30
A	see column 5, line 1-10; claims 1-3,5-8 see column 5, line 38 - column 6, line 12 see column 6, line 39-52 see column 4, line 36-49 see column 3, line 11-21	9-16, 18-25

X	US 5 502 058 A (VIRGINIA COMMONWEALTH UNIVERSITY) 26 March 1996	17,26, 27,29,30
A	see column 1, line 14-43; claim 1 see column 1, line 54-60 see column 2, line 43-55 see column 4, line 8-22 see column 4, line 43-48 see column 4, line 62 - column 5, line 33	1-16, 18-25

X	WO 96 27386 A (HOUGHTEN PHARM INC) 12 September 1996 see page 12, line 10-14; claim 1; examples 5,7,11,16 see page 25, line 17 - page 26, line 25	9,10, 12-14, 16,17, 26,27,29
A	see page 29, line 1-23; claims 1-17,32,39	1-8, 18-25

X	WO 95 24382 A (G.D. SEARLE & CO) 14 September 1995	17
A	see page 3, line 14; claims 1,10,11 see page 7, line 3	26-30

X	WO 95 11014 A (G.D. SEARLE & CO) 27 April 1995	17
A	see page 3, line 20; claim 5	26-30

A	US 5 256 669 A (ASKANAZI JEFFREY ET AL) 26 October 1993 see column 2, line 1-60 see column 3, line 60 - column 4, line 7 see column 5, line 4-21 see column 5, line 42-47; claims; examples 1,2	1-30

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INTERNATIONAL SEARCH REPORT

national Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 19440 A (WELLCOME FOUNDATION LTD) 27 June 1996 see page 2, line 15 - page 3, line 14; claim 1 see page 3, line 16 - page 4, line 6 see page 8, line 1-16 see page 13-15 see page 16, line 15 - page 17, line 11 see page 17, paragraph 2	1,3-9, 11-18, 20-26, 28-30
A	EP 0 713 704 A (HOECHST AG) 29 May 1996 see page 10, line 48; claim 1 see page 2, line 40-44 see page 2, line 29-32	9-17, 26-30
A	WO 93 05775 A (US HEALTH) 1 April 1993 see page 3, paragraph 2; claims 1,4,5,10-14 see page 11, paragraph 2 - page 12, paragraph 1	9-16, 26-30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/00006

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark : Although claims 1-25 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

mational Application No
PCT/US 98/00006

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Information on patent family members

...rnational Application No
PCT/US 98/00006

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